International Journal of Bio-Technology and Research (IJBTR)
ISSN(P):2249-6858; ISSN(E):2249-796X
Vol. 7, Issue 3, Jun 2017, 29-36

TRANS STELLAR
•Journal Publications •Research Consultancy

Original Article

FEATHER DEGRADATION AND KERATINASE PRODUCTION BY *BACILLUS* SP. AND *LACTOBACILLUS* SP.

PUNIKA CHAISEMSAENG, ARTIT SABU, SUPASET ANSANAN & SUJAREE SIRISAN

Department of Science and Mathematics, Kalasin University, Muang Kalasin, Kalasin Province, Thailand

ABSTRACT

The aim of this research was to investigate the potential of chicken feather degradation by Bacillus sp. and Lactobacillus sp. The bacteria were grown with 43 °C and pH 7.5 in basal medium, the growth cell of bacteria, dry weight and percentage of the feather were measured. Both strains could degrade part of the feather at 5 and 10 days. The bacteria growths were monitored by turbidity method. The growth of cell showed Bacillus sp. had highest of growth, cell at 10 days. Whole feather, dry weights of both strains was related to a percentage of the feather degradation. These found, Lactobacillus sp. resulting great in feather, dry weight and percentage of feather degradation, higher than that the Bacillus sp. This strain showed the feather, dry weight at 67.67 grams and partial degradation of the feather at 32.33% after 10 days of incubation. Keratinolytic activity assay were shown as 50.36 and 60.92 U/ml from Lactobacillus sp. and Bacillus sp., respectively. This is very interesting; because of Lactobacillus sp. showed great of a feather degrading percentage, but secreted the enzyme less than Bacillus sp. strain. This strain has been a little reported for feather degradation and we indicated that the strain could be strong to degrade feather of the chicken and can be a highly useful bacterium for feather meal production and in leather industry.

***KEYWORDS: Feather Degradation, Keratin, Keratinolytic Production & Bacillus* sp. *& Lactobacillus* sp.**

Received: Apr 29, 2017; **Accepted:** May 21, 2017; **Published:** Jun 05, 2017; **Paper Id.:** IJBTRJUN20174

INTRODUCTION

Background/Objectives and Goals

Poultry, especially chickens in Thailand have developed in a sequence from the home to industry. Thailand is considered as the largest chicken exporter in Asia (Eksittikul and Kudan, 2015). The process of this chicken is generated in abundant amounts of feather as byproducts. Each year, poultry production waste from processes such as blood, bone, feathers and chicken, especially chicken feather waste expected up to 50,000 - 80,000 tons per year. The chicken feathers contain over 80-91% crude protein in a form of β-keratin (Lakshmi et al., 2013; Kainoor and Naik, 2010). β-keratin is the generality composition in feathers which defines as hard to digest animal protein. Keratin is insoluble and not degradable by protease enzyme, because of a high degree of cross-linking by cysteine disulfide bonds and hydrogen bond interaction (Park and Son, 2009). In addition, keratin in feathers can be degraded by keratinolytic enzyme from some microorganisms. Currently, feather treated with microbial keratinase is attaching wide attention with several applications. In previously has been reported for keratinolyticbacteria**;** for example, *Aspergillus* sp., *Alternaria Radicina*, *Trichus spirals*, *Streptomycespactum*, *S. albus*, *S. thermoviolaceus*, *Bacillus* sp. (Suntornsuk and Suntornsuk, 2003). Keratinase treated the feathers is increasingly respected as a source of dietary protein in food and feed supplement. Because of, the feather wastes are utilized on a basis as a dietary protein supplement for animal feed. Previously used, feather wastes were

cooked and chemical treated to digestion. In the present day, the use of some microorganisms represent in an alternative way to improve the nutrient value of the feathers. It has already been demonstrated that the feathers digested by *Bacillus licheniformis* strain PWD-1 or *Bacillus* sp strain FK 46 have nutritional feathers for feed used similar to soybean protein (Williams et al., 1991; Suntornsuk and Suntornsuk, 2003). The feathers degrade by *Bacillus* could be replace as 7% in nutritional feed and dietary protein for growing animals (Qdetallah et al., 2003).

The keratinolytic bacteria showed a potential to be utilized for feather degradation and dietary protein supplement in animal feed stuffs. The feather protein has shown an excellent source of metabolize protein and microbial keratinase enhance the digestibility of feather keratin (Tamilmani et al., 2008). Keratinase is possibly used for supplementation of enzyme in a small amount in the diet, may improve the feed digestibility and promote the growth of broiler chicken (Poovendran et al., 2011). Keratinolytic enzymes may have important uses in a biotechnological process involving keratin containing wastes from poultry through the development of non pollution process (Park and Son, 2009). In this paper, we describe the primary study and the investigated potential for feather degradation of the chicken and enzyme activity by keratinolytic bacteria.

METHODS

Source of Keratin

Chicken feathers (whole feathers) were collected from chicken slaughter, Lub village, Muang Kalasin district, Kalasin province, Thailand. The feathers were washed with the tap water and air dry. Feathers were incubated with hot air oven at 60°C for 48 h or until still dry weight. Then, these were sterilized again with autoclave (121°C, 15 pond and 20 minutes). They were dried at 60°C to constant weight and kept at 4°C until used.

Microorganisms

The strains *Bacillus* sp. And *Lactobacillus* sp. used in this study was isolated in the department and maintained in a NA slant at 4°C until used. The bacterium was grown in basal medium (0.1% $MgCl_2$, 0.12% K_2HPO_4, 0.22%KH_2PO_4, 0.17% NH4Cl, and 0.05% NaCl) and 0.1 g/l of yeast extract and pH was adjusted to 7.5 to prepare for cultivation conditions.

Cultivation Conditions

This research was tested by completely randomized design (CRD), it composed; the feather treated with *Bacillus* sp. and treated with *Lactobacillus* sp. Each experiment was carried out in sterile 500 ml glass bottle that contained 100 grams of the whole feather. 100 ml of bacteria culture at $6x10^8$CFU/ml (O.D.= 0.1) was added to the feather and then vigorously mixed with shaker incubator at 120 rpm for 24 h. After this, the culture was incubated at 43 °C for 5 and 10 days. The fermentation broth at 5 and 10 days were sampled for analysis of bacterium growth. Feathers were analyzed for dry weight and percentage of the feather degradation.

Analysis

Bacterial growth in each treatment was determined by O.D. at 600 nm. The feathers were harvested by filtration with Whatman number 4 filter paper, washed with sterile distill water and air dried at 60°C to constant weight. The percentage of feather degradation was calculated from the differences in the residual feather, dry weight compared with the control (feather without bacterial inoculation).

Preparation of Keratin Solution

Soluble keratin was prepared from chicken feathers by method of Wawzkiewcz et al. (1987). Ten grams of native chicken feathers were washed and suspended in 500 ml of dimethyl sulfoxide with solubilized by heat at 100 °C for 1 h. Next, this soluble was precipitated by 1000 ml of cold acetone at -20 (C for 1 h. Soluble was centrifuged at 10,000xg for 10 min, washed with distill water and dried at 40 °C in vacuum dryer. One gram of precipitate was dissolved in 20 ml of 0.05 Mol/L of NaOH and adjusted the pH to 8.0 with 0.1 Mol/L Tris-HCL. The soluble was re-suspended with 200 ml of 0.05 mol/L of Tris-HCL buffer (pH 8.0) and used 0.5% (w/v) as substrate for keratinase activity determination.

Keratinolytic Activity Determination

Keratinase activity was assayed by Zheng et al. (2008). This method was used 1.0 ml of crude enzyme diluted in 0.05 mol/l of Tris-HCL buffer (pH 8.0) and incubated with 1 ml of keratin solution at 50 °C for 10 min. The reaction was stopped with 2 ml of 0.4 mol/L trichloroacetic acid. Later, the mixer was centrifuged at 1500xg for 30 min and the supernatant was kept. The absorbance of the supernatant was determined at 280 nm (UV-1800, Shimadzu). The control was prepared by incubating the enzyme solution with 2.0 ml of TCA without keratin solution.

One unit (U/ml) of keratinase activity was defined as an increase of the observance of 280 NM with control for 0.01 per minute, under the conditions and calculated by the following equation:

$$U = \frac{4 \text{ x } n \text{ x } A280}{(0.01 \text{ x } 10)}$$

Where, n is the dilution rate, 4 is the final reaction volume (ml) and 10 is the incubation time (min).

Statistical Analysis

Data analysis of bacterial growth, the dry weight of whole feather, percentage of feather degradation and keratins activity were done by using comparison of means with one-way ANOVA of SPSS statistics (version 19.0), SPSS Inc., Chicago, IL USA). The least significant difference (LSD) tested at $P<0.05$.

RESULTS

The strains *Bacillus* sp. and *Lactobacillus* sp. showed good efficacies to degrade the feather in the medium. Both strains presented great to grow and increased of cells from 5 and 10 days. Keratinolytic bacteria strain Bacillus sp. Can grow from 0.1 ($O.D._{600}$) to 0.187 and 0.414 at 5 and 10 days, respectively. In addition, *Lactobacillus* sp. Showed the same result in growth of cell at 0.150 (5 days) and 0.358 (10 days) (Table 1). Both trains showed no differences in growth at the same time, but, they can grow rapidly and showed significant differences from 5 to 10 days ($P<0.05$). So, *Bacillus* sp. showed highest a maximum of growth (0.414) at 10 days (Figure 1). The feather, dry weight of both bacteria demonstrated decreasing, as the time course was increasing. At final time incubation (10 days), *Lactobacillus* sp. Played highest of the feather, dry weight lost (67.67 g) and showed significant differences of the dry weight at 5 days (72.33 g) ($P<0.05$). However, *Bacillus* sp. Presented trend to decrease of feather, dry weight, but showed no significant difference from5 to 10 days as 73.12 g and 71.83 g respectively, (Figure 2).

Table 1: **Keratinolytic Bacterial Growth, Dry Weight and Feather Degradation at 5 and 10 Days**

Data	5 Days		10 Days	
	***Bacillus* sp.**	***Lactobacillus* sp.**	***Bacillus* sp.**	***Lactobacillus* sp.**
Bacterial growth (CFU/ml)	0.187	0.150	0.414	0.358
Dry weight (g)	73.12	72.33	71.83	67.67
Feather degradation (%)	26.88	27.67	28.17	32.33

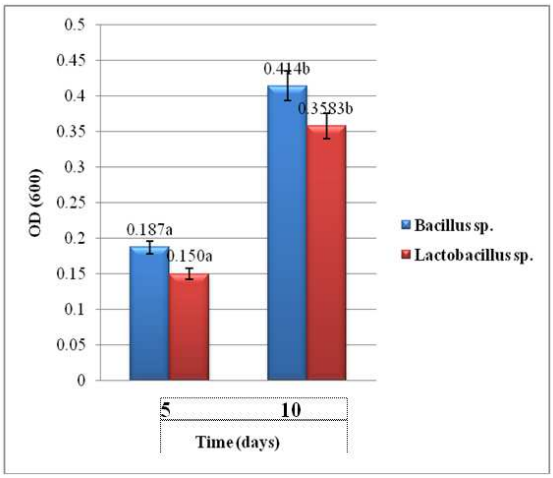

Figure 1: The Growth of *Bacillus* sp. and *Lactobacillus* sp. were Measured (O.D. 600_{nm}), after Incubated at 43°C for 5 and 10 Days. Bars Represented the Means ± Standard Error. The Letters on the Top of Each Bar Indicate Significant Difference (*P<0.05*)

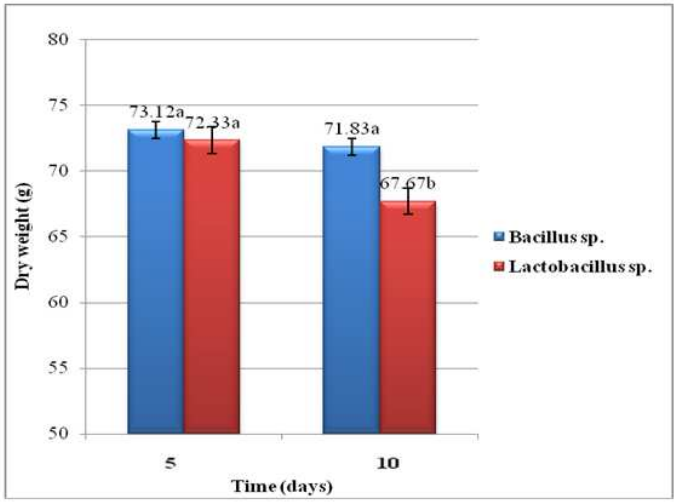

Figure 2: Dry Weight of Whole Feather of the Chicken after Incubated at 43°C for 5 and 10 Days that Inoculums with *Bacillus* sp. and *Lactobacillus* sp. Bars Represented the Means ±Standard Error. The Letters on the Top of Each Bar Indicate Significant Difference (*P<0.05*)

The percentage of feather degradation by *Lactobacillus* sp. and *Bacillus* sp. are shown in Figure 3. Partial degradation was observed after incubation at 43 °C for 5 and 10 days. The strain *Lactobacillus* sp. Showed highest percentage of feather degradation as 27.67% and followed by *Bacillus* sp. as 26.88% at 5 days. They showed no differences of the percentage of the feather degradation ($P>0.05$). Similar results in percentage of feather degradation at 10 days showed the highest percentage at 32.33% from *Lactobacillus* sp. and followed by 28.17% from *Bacillus* sp. strain.

The percentage of feather degradation of both strains presented showed significant differences in 10 days (P<0.05). From the results, it is indicated that *Lactobacillus* sp. the strain had the efficacy to degrade the whole feather of the chicken more than *Bacillus* sp. strain. Keratinolytic activity assay were shown at 60.92 U/ml by *Bacillus* sp and 50.36 U/ml from *Lactobacillus* sp. (Figure 4).

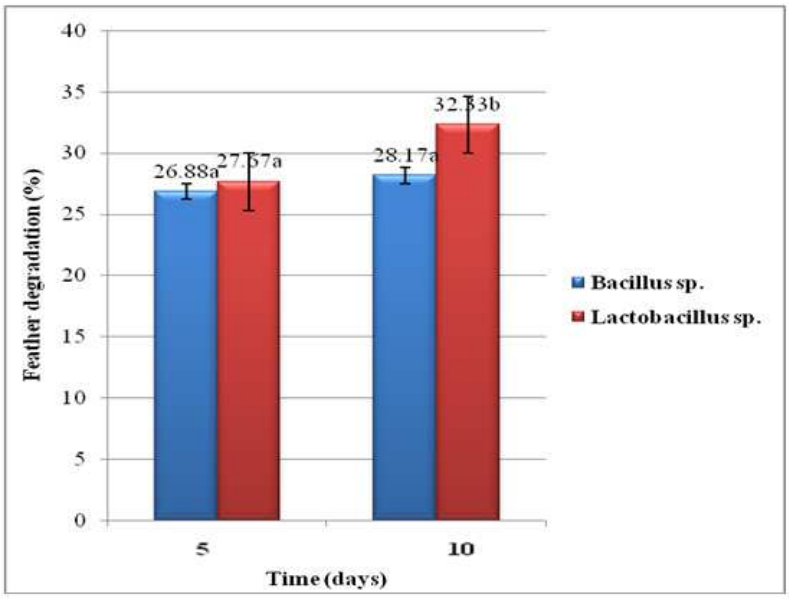

Figure 3: Feather Degradation by *Bacillus* sp. and *Lactobacillus* sp. after Incubated at 43°C for 5 and 10 Days. Bars Represented the Means ±Standard Error. The Letters on the Top of Each Bar Indicate Significant Difference (*P<0.05*)

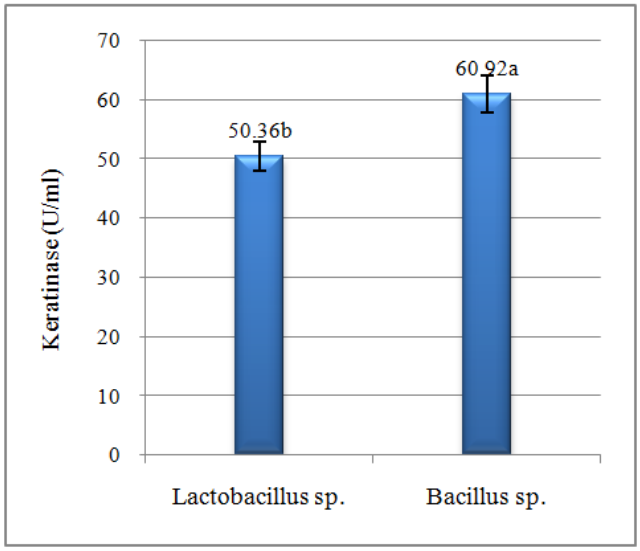

Figure 4: Keratinolytic Activity (U/ml) of *Bacillus* sp. and *Lactobacillus* sp. Activity was Measured after Growth for 48 h at 28 °C in the Medium Contain Keratin Substrate with the Cell 10^7 CFU/ml of Each Strain. Bars Represented the Means ± Standard Error. The Letters on the Top of Each Bar Indicate Significant Difference (*P<0.05*)

CONCLUSIONS AND DISCUSSIONS

The growth of cell, dry weight and percentage of feather degradation of the chicken by *Bacillus* sp. and *Lactobacillus* sp. were measured at an incubation conditionsat43 °C for 5 and 10 days and pH 7.5. Both species typically are mesophilic, can grow well and occurred in a broader temperature range of 15-45 °C (Park and Son, 2009). This report is agreeable with Kim et al. (2001) who presented that, the best temperature in the feather medium for production of keratinolytic enzyme by degrading bacilli (*B. licheniformis* and *B. brevis*) was between 40-45 °C. This research used the pH of the basal medium at 7.5. The *Bacillus* species can grow rapidly at the pH 7.5-8.8. These cultivation conditions were optimized to stimulate the bacteria growth for produce hydrolytic enzymes (Park and Son, 2009). The efficacies of both strains degrade whole feather chicken were observed the percentage of feather degradation and dry weight. This research found both strains can degrade part of the whole feather. The result showed all of the feathers were partial degraded after 10 days cultivation and not completely degradation. Some research reported about the time course for feather degradation such as *B. megaterium* F7-1 can degraded partial of the chicken feather, after 4 days and completely degraded after 7 days of cultivation (Park and Son, 2009), and *B. licheniformis* PWD-1 degraded the chicken feather completely at 50 °C in 10 days (Williams et al., 1991). In this research, it is found that the feather cannot degrade completely in 10 days, because the enzyme mechanism could be less partial of disulfide bond and complex of cysteine amino acid. However, this research showed the best percentage of feather degradation as 32.33% that closely with Suntornsuk et al. (2005) reported as 51% of feather degradation at 10 days. The feather protein was degraded by curtains enzyme and utilized as nutrient for bacterial growth. The important mechanism is that, known keratinase enzyme activities that are produced by keratinolytic microorganisms. Keratinase are enzymes that can hydrolyze both native and denatured keratin. Previous study has shown that the synthesis of extracellular keratinase is constitutive and inducible by feather substrate (Park and Son, 2009). Feather was the optimal substrate for keratinase production (Zheng et al., 2008. Several researches reported *Bacillus* sp. is a good producer of keratinase; *Bacillus* sp. JB99 (Kainoor and Naik, 2010), *Bacillus* sp. FK46 (Suntornsuk and Suntornsuk, 2003), *B. pumilis*, *B. cereus* and *B. subtilis* (Kim et al., 2001), *B. licheniformis*(Poovendran et al., 2011; Tamilmani et al., 2008; Radha and Gunasekaran, 2009). However, our study found *Lactobacillus* sp. had a great potential to degrade the whole feather with highest percentage of feather degradation. This result is very interesting, because this strain has been a little reported for feather degradation of the chicken but, it has been reported that communicate in gut of bird; *L. crispatus*, *L. reuteri* and *L. salivarius* and these species can hydrolyzed the keratin in gut (Meyer et al., 2012).

In the further study, the difference of the carbon sources and the optimal conditions of bacterial cultivation on keratinase production need to be measured for confirming the mechanism of feather degradation by both bacteria.

ACKNOWLEDGEMENTS

I would like to thanks Assist. Sujitra Saravit for discussions and suggestion on this study and thank to the students from Department of Animal Science, Kalasin University for helps to do the complete research. This research was funded by Kalasin University, Thailand.

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